Determination of Sucrose Content of Milk by Polarimetry

**Purpose:** Determination of sucrose content of milk and milk product

The sucrose content is total amount of unaltered sucrose in the milk product determined by the procedure specified in the International Standard ISO 2911. It is expressed as a percentage by mass.

**Principle:**
This analysis is based on the fact that a beam of polarised sodium light is rotated when falling through a sugar solution. The angle of rotation is specific for a certain kind of sugar and is in direct proportion with the sugar concentration and the distance traveled. This distance is fixed by the standard tube of 20 cm.

The milk solution is treated with ammonia to bring mutarotation of lactose to final equilibrium and is neutralized by acetic acid.

The neutralized milk solution is clarified by successive addition of zinc acetate and potassium hexacyanoferrate (II) to precipitate protein and fat in milk, and next step is filtration.

One part of the filtrate, the rotation angle of sucrose is measured. Another part of the filtrate is measured after inversion of the sucrose present into fructose and glucose by mild hydrochloric acid. Glucose and fructose both have a different specific rotation from original sucrose. From the difference between these two measurements the sucrose percentage can be calculated.
Procedure

Preparation of sample

-> Container of milk (sample) should be shaken and scrolled a few times.
-> Open the container and stir the sample carefully by glass rod.
-> Transfer the sample to the glass bottle and close it.

If the sample is too old or too thick:

-> The sample has to be warmed up 40°C in the water bath (6.5) for about 2 hours.
During the warming time, every 15 minutes, take the sample out and shake it well.
Transfer the sample to glass bottle and cool it down to room temperature.

Determination

-> Weigh exactly about 40g of sample into beaker (6.8)
-> Add 50ml of hot water (80-90°C) and mix well
-> Transfer the mixture quantitatively to a 200ml volumetric flask (6.2), rinsing the beaker with successive quantities of water at 60°C until the total volume is between 120 and 150ml. Mix and to cool to about 20°C.
-> Add 5ml NH₃ solution (7.3), mix and allow to stand for 15 min.

Note: The exact amount of CH₃COOH required is determined by titration of the NH₃ solution, using bromothymol blue as indicator.

-> Add 12.5 ml of Zn(CH₃COO)₂ (7.1) solution and then 12.5 ml of K₄Fe(CN)₆ (7.2) solution and mixing gently by rotating the flask
-> Bring the content of the flask to 20°C and dilute to the mark with water at 20°C.

Note: Up to this stage, all mixing shall be done by rotating the flask rather than by shaking to avoid the formation of air bubbles

-> Close the flask with a dry stopper and mix thoroughly by vigorous shaking.

-> Allow the flask to stand for 15 min. and filter through a dry filter paper (6.13), rejecting the first 25 milliliters of filtrate. Collect the remaining clear filtrate in the clean and dry erlenmeyer (6.8)

-> Close the Erlenmeyer and cool to 20°C in the water bath (6.6), make sure that the contents are below the level of the water in the water bath (Solution A)

-> Pipette (6.11) 40ml of the filtrate (Solution A) into a 50ml volumetric flask (6.3), add 6ml HCl (7.5).

-> Place the flask in the water bath at 60°C (6.4) for 15 min, taking care that the entire bulb of the flask is immersed. Mix by rotating the flask during the first 5 min.

-> Cool the flask to 20°C in water bath (6.6), and dilute the mark with water at 20°C, mix and allow standing for 1 hour at this temperature. (Solution B)

Measurement

1. Direct polarization
   Fill clean a 100 mm long polarimeter tubes with solution A at 20°C. Place the filled sample tube in the polarimeter and record the optical rotation (A).
2. Do the same way for the solution B and record the optical rotation (B).
Calculation

Use the following formula

\[ S = \frac{A - 1.25B \cdot V - \Delta V \cdot V}{Q \cdot V \cdot L \cdot m} \]

- \( S \): % sucrose
- \( m \): weight of sample in gram
- \( A \): the reading of A solution
- \( B \): the reading of B solution
  
  + If the invert polarization is measured at a temperature \( t \), other than \( 20 \pm 0.2^\circ C \), the value of \( B \) should be multiplied by:
    \[
    (1 + 0.0037(t - 20))
    \]
- \( L \): the length, in decimeters, of the polarimeter tube
- \( Q \): the inversion division factor (given in 9.2 below)
- \( V \): the volume, in milliliters, of diluted sample before filtration
- \( DV \): the correction, in milliliters, for the volume of the precipitate formed during the clarification

\[
\Delta V = \frac{m}{100} \left(1.08F + 1.55P\right)
\]

Where \( F \): % fat in the sample, \( P \): % protein in the sample.

\[ S = \frac{A - 1.25B \cdot V - 200 - \Delta V}{Q \cdot 2 \cdot m} \]

Recommended polarimeter tube

=> Schmidt+Haensch

Stainless steel tube with funnel and riser, equipped with
Connections for water circulator and with integrated
temperature probe
Additional calculation:

--> Value of the inversion division factor Q

The following formula gives an accurate value for Q, where the light source is sodium light, and rotation is measured in angular degrees

\[ Q = 0.8825 + 0.0006(C - 9) - 0.0033(t - 20) \]

Where

- \( C \): the percentage of total sugars in the inverted solution according to the polarimetric reading.
- \( t \): the temperature, in degrees Celsius, of the inverted solution during the polarimetric reading.

Remarks:

1. The percentage of total sugars \( C \) in the inverted solution may be calculated from the direct reading and the change on inversion in the usual manner, using the usual values for the specific rotations of sucrose, lactose and invert sugar. For normal condensed milk, the correction 0.0006\((C - 9)\) can be neglected because \( C \) very close to 9.

2. Variation in temperature from 20°C makes little difference in the direct reading, but variation of more than 0.2°C in the invert reading necessitates a correction. The correction factor given to \( B \) in only accurate between 18 – 22°C

Recommended polarimeter

--> Schmidt+Haensch Polarimeter Polartronic N / M series or Unipol L series

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